RECEIVED
CENTRAL FAX CENTER

MAR 1 8 2005

Attorney Docket No.: 4324.224-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hansen et al. Confirmation No: 2312

Serial No.: 09/467,368 Group Art Unit: 1652

Filed: December 21, 1999 Examiner: Rao, M

For: Animal Feed Additives

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dan Robert Pettersson, do hereby state and declare that

1. I am the same Dan Robert Pettersson who executed a Declaration under 37 C.F.R. 1.132, which was submitted on September 4, 2004 ("Original Declaration").

2. The experiments described in my Original Declaration were carried out under my direction and supervision.

3. As described in paragraph 2 of my Original Declaration, two xylanases were tested in feed trial to measure apparent metabolisable energy (AME). One xylanase was a *Thermomyces lanuginosus* xylanase having an amino acid sequence of SEQ ID NO: 2 of the present application, and the other was a xylanase of Family 10 xylanase derived from *Aspergillus aculateus* (xylanase 2 of WO 94/21785). The enzymes were dosed at 200 FXU/kg feed composition. The results of the trial are reproduced in the following tables:

Enzyme	AME (MJ/kg)	FCR (feed:gain)	Excreta moisture (%)
No Enzyme	13.65	2.16	77.1
Enzyme derived from			
Thermomyces lanuginosus	14.50	1.97	73.4
Aspergillus aculateus	14.15	2.02	75.0

	Viscocity, cP		
Enzyme	Duodenum	Jejunum	Ileum
No Enzyme	3.28	7.99	23.31
Enzyme derived from			
Thermomyces lanuginosus	2.16	4.06	6.77
Aspergillus aculateus	2.31	3.32	7.75

4. I wish to clarify the statement in paragraph 4 of my Original Declaration that, "The results demonstrate that animal feeds comprising the *Thermomyces lanuginosus* xylanase results in significantly better feed utilization than animal feed compositions comprising the *Aspergillus aculateus* xylanase." The treatment with the *Thermomyces lanuginosus* xylanase resulted in a statistically significant reduction in Feed Conversion Ratio ("FCR", which means a better feed utilization) as compared to the control (*i.e.*, no enzyme). In contrast, the treatment with the *Aspergillus aculateus* xylanase did not result in a statistically significant reduction in FCR relative to the control. Accordingly, the *Thermomyces lanuginosus* xylanase performs better than the *Aspergillus aculeatus* xylanase, because a statistically significant result is obtained only for the *Thermomyces lanuginosus* xylanase.

5. The results furthermore show, with statistical significance, that both xylanases have a strong effect on the viscosity of the digesta, as compared to the control. As regards the *Thermomyces* xylanase, this is surprising and unexpected, because one of ordinary skill in the art would expect a xylanase of family 10 such as the *Aspergillus* xylanase to reduce viscosity to a greater extent than a xylanase of family 11 such as the *Thermomyces* xylanase. This is because it is well known that xylanases of family 11 have a kinetic preference for insoluble xylans, and therefore would not be expected to reduce viscosity which is caused by soluble xylans. It is also well known that there is a correlation between animal feed utilization and digesta viscosity, generally a lowering of the viscosity is beneficial for the feed utilization (see, e.g., G. S. Burnett, "Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation," British Poultry Science, 7:55-76, 1966).

6. In summary, these results show that animal feeds comprising the *Thermomyces lanuginosus* xylanase result in a statistically significantly improved feed utilization. A similar

conclusion cannot be drawn as regards animal feeds comprising the *Aspergillus aculeatus* xylanase. Furthermore, the *Thermomyces lanuginosus* xylanase has a statistically significant influence on the viscosity of the digesta, which is highly surprising, given the fact that family 11 xylanases are not recognized to be strong viscosity reducers. These results are surprising and unexpected.

7. Furthermore, the results in Examples 6 and 8 of the instant application demonstrate that animal feed compositions comprising a thermostable xylanase of Family 11 have a significantly better feed utilization than animal feed compositions comprising a commercial xylanase product, *i.e.*, BIO-FEED PLUS CT. In particular, Example 6 demonstrates that, surprisingly, a *Thermomyces lanuginosus* xylanase of the present invention has significantly improved properties in reducing wheat viscosity in vitro than a prior art xylanase (a commercially available multicomponent enzyme preparation derived from *Humicola insolens*). See also Figure 4, which shows a much higher efficiency of the xylanase of the invention as compared to the prior art xylanase per unit dosage with respect to viscosity reduction. Using for example 1.29 activity units per gram of wheat of each of these xylanases, the relative viscosity when using the xylanase of the invention is about 40%, whereas it is only about 75% when the prior art xylanase is used. As described in the example, there is a close correlation between reduction of viscosity and improvements in chicken feed conversion efficiency. These results are surprising and unexpected.

8. Furthermore, in Example 8, Applicants have compared the digestibility of animal feeds comprising a thermostable xylanase of Family 11 ("A" and "B") and the digestibility of an animal feed comprising Bio-Feed Plus ("C"), a commercially-available xylanase preparation derived from *Humicola insolens*. The results show that the use of Bio-Feed Plus at a dose of 400 FXU/kg gave a % fat digestion of 72.4, whereas the animal feeds comprising a xylanase of the present invention gave a % fat digestion in the range of 72.1-74.3 even though the xylanase was dosed at 100 or 200 FXU/kg (one quarter or one-half, respectively, of the Bio-Feed Plus). These results demonstrate that animal feeds comprising a thermostable xylanase of Family 11 have a significantly better digestibility than an animal feed comprising Bio-Feed Plus. Since the demonstrated superior property is not predicted by the prior art, these results are surprising and unexpected.

9. The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to

be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize any patent issuing thereon.

Signed this 18th day
of March 2005

Dan Pettersson

Dan Robert Pettersson